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Contents

Serotonieric system - SNP`s and epigenetic markers - in the control of appetite and satiety

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Sprachen: Deutsch Muttersprache, Englisch fließend
EDV: Microsoft Office Produkte, sicherer Umgang mit PC und Internet, SPSS

Declaration

Herewith I affirm that I have written this thesis on my own. I did not enlist unlawful assistance of someone else. Cited sources of literature are perceptibly marked and listed at the end of this thesis. The work was not submitted previously in same or similar form to another examination committee and was not yet published.

_____ _____

Date Signature

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Abstract

Are you aware of your inner power system?

An immense power is shaping your inner world – your experience and conception of life. You are altering your brain chemistry, manipulating your neurochemical profile and affecting your body's physiology every day by what you do and don't eat, what you think, and how and where you spend your time. Through your daily behavior and the environments in which you live, you create and shape your biochemical profile. This in turn is reflected in your emotions, energy, thoughts, actions, and psychological condition that either bring out your peak performance or that block your optimal functioning or even can cause sickness.

The unbalanced homeostasis of serotonin in the body is often attributed to anxiety, depression, panic attacks, insomnia, obesity, fibromyalgia, eating disorders, chronic pain, migraines, and alcohol abuse.

My thesis "Serotonieric system - SNP`s and epigenetic markers - in the control of appetite and satiety" describe not only a very complex subject in itself but also how it affects and interacts with a huge number of inner systems and pathways.

The thesis is structured in 5 chapters:

The first two chapters provide an overview over the most recent and significant research and contain important definitions and information.

The first chapter is concerned with the genetic and epigenetic markers which are associated with obesity and how genetic and epigenetic "talk" to each other.

The second chapter reviews our scenery of investigation: the serotonic system: how is it influenced in regard to weight gain or loss. Here, not only the single receptors are closely observed but also other possible factors that potentially have an impact on appetite or satiety.

The third and forth chapters contain the practical part of the thesis. They provide the rationale and procedures of my argumentation. Consequently, the third chapter discusses the methodology of meta-analysis, its potential, complexity and limitation. The forth chapter is the meta-analysis of the HTR2C polymorphism (-759 C/T) to obesity itself. The result of the analysis can be summarized in the realization that the genetic variation alone only has a borderline significance to obesity. Therefore other possibilities

must be considered in further research, for instance a special selection of different polymorphisms or, most plausibly, the effect of epigenetic markers and environmental triggers that influence us during our entire life.
For that reason the fifth chapter is entirely dedicated to epigenetic and again, very shortly, genetic markers at the serotonic receptors and environmental exposure, introducing also personal life style choices that influence our eating behavior.
Epigenetic research - still at its very early stage - opens a vast and exciting new dimension. A vast amount of scientific research is required to begin harnessing its staggering potential.

1. Introduction

Overweight and obesity has clearly become a major clinical and public health burden worldwide. In our society which is sometimes shallow and mainly focused on external appearance, overweight and obesity are met with personal and social criticism. But it is the higher risk of type-2-diabetes (T2D), cardiovascular disease, cancer and mortality [Flegal KM et al., 2007; Pischon T et al., 2008] correlated with the obese phenotype that is the main problem and center of acute research. Despite all efforts, prevailing undertaken measures to prevent obesity such as exercise, diet, education, drug therapy and surgery are failing.

To control obesity and improve prevention and therapy, we have to study and apprehend its pathway and mechanisms.

In this paper I focus on the identification of genetic variations influencing obesity and recent evidence that could connect epigenetic events with obesity.

Until today, more than 40 genetic variants have been identified as being connected with obesity and fat distribution. However, since these findings do not completely elucidate the heritability of obesity, other forms of variation, for example epigenetic marks, must be considered.

2. Genetic variations and obesity

Until 2006 the main concepts used to identify common variants shaping the obese phenotype were association studies within ‘candidate’ genes using case–control samples or parent–offspring trios or hypothesis-free genome-wide linkage mapping in families with multiple obese subjects. These approaches suffered from being underpowered because reasonable susceptibility models, as linkage are best placed to track down variants with high penetrance. Selecting credible candidates, the candidate-gene association approach have historically been compromised. Selection was usually founded on hypotheses about biological mechanisms putatively associated with obesity pathogenesis, however, as the mechanism of much of the genome is poorly understood, it remains almost impossible to make well-founded decisions. In addition, the candidate-gene studies were far too often executed in sample sets too small to achieve confident identification

of variants which are now understood to be realistic in regard to their range of effect sizes.

Accordingly, over the last two decades, efforts in detecting and replicating genetic variants predisposing individuals to normal forms of obesity were largely described by limited success and slow progress. This is in acute contrast to the effective gene identification in syndromic and monogenic forms of obesity [Rankinen T, et al., 2006]. The "Human Obesity Gene Map" provides a brilliant overview of this*:* it contains 11 single gene mutations, 50 loci related to Mendelian syndromes, which are listed as relevant for human obesity*,* 127 candidate genes and 244 knockout or transgenic animal models, of which slightly fewer than 20% are repeated by 5 or more studies [Rankinen T, et al., 2006]. A total of 253 quantitative trait loci (QTL), for diverse obesity-related phenotypes, have been announced from 61 genomewide linkage scans and of these, only ~20% are assisted by more than one study [Rankinen T, et al., 2006].

In the past three years it has become possible to conduct hypothesis-free genom wide association (=GWA) testing in samples of effective size to generate persuasive association outcomes. The dawn of the GWA studies was the result of three elements.

1. Human genome sequence: enabled cataloguing genome-sequence variation
2. International HapMap Consortium (http://www.hapmap.org) [A haplotype map of the human genome. Nature 2005]: it taught us that, in non-African-descent populations, extensive correlations (linkage disequilibrium, LD) between neighboring single nucleotide polymorphisms (SNPs) constrain the number of independent genetic tests required to survey the genome, such that ~80% of all common variation can be sampled using ~500 000 carefully selected SNPs [Barrett JC et al.,2006; Pe'er I et al. 2006].
3. Novel genotyping methods: challenges of massively parallel SNP-typing at high accuracy and low cost [Fan JB et al., 2006].

The undeniable success in identifying loci for common forms of obesity (as defined by anthropometric measures: BMI, WC and/or WHR) with the GWA approach is shown in table 1: 15 'high-density' GWAs have yielded over 50 loci associated with obesity.

Table 1: Overview of GWA scans or meta-analysis for different obese phenotypes

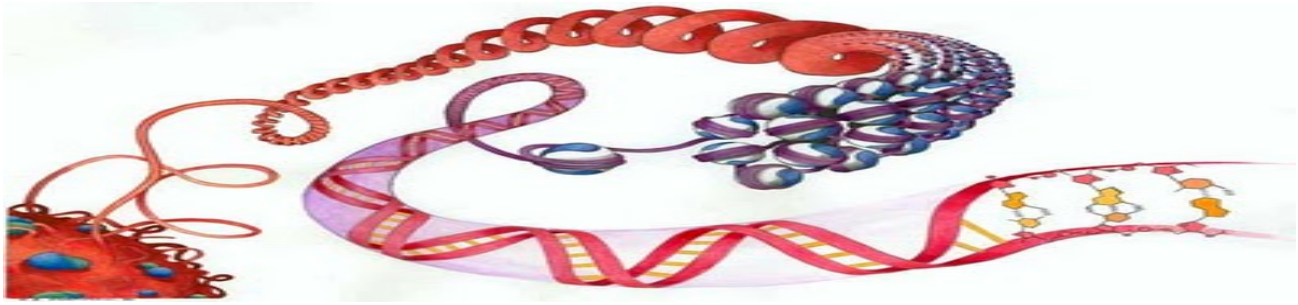
Reference	Study name (if any)	Number of samples in discovery cohort	Ancestry of discovery cohort	Phenotype
Frayling et al.	WTCCC	1924	Europeans	BMI – quantitative analysis
Scuteri et al.	Sardinia	4741	Europeans	BMI – waist circumference (WC) quantitative analysis
Loos et al.		16 876	Northern European	BMI – quantitative analysis
Chambers et al.	LOLIPOP	2684	Indian Asians	Insulin resistance and related quantitative phenotypes
Willer et al.	The GIANT consortium	32 387	Europeans	BMI – quantitative analysis
Thorleifsson et al.	DeCODE	37 347	Europeans + African Americans	BMI – quantitative analysis
Meyre et al.		1380 and 1416 age-matched normal-weight control	Europeans	Early onset and morbid adult obesity
Cotsapas et al.		775 cases and 3197 unascertained controls	Europeans	Extreme obesity/BMI
Scherag et al.		453 extremely obese youngcases and 435 healthy lean controls	Europeans	Extreme obesity/BMI
Lindgren et al.	The GIANT consortium	38 580	Europeans	WC and waist:hip-ratio (WHR) – quantitative analysis
Heard-Costa et al.	The CHARGE consortium	31 373	Europeans	WC – quantitative analysis
Speliotes et al.		123 865	European	BMI – quantitative analysis
Cho et al.	KARE	8842	Asian	BMI, WHR – quantitative analysis
Heid et al.	MAGIC	77 167	European	WHR – quantitative analysis

The most common known gene associated to obesity is perhaps the FTO [Frayling TM et al. 2007], which was identified during a GWA of T2D [Zeggini E et al., 2007]. ~1% of BMI heritability is explained by the association of the FTO region and obesity. This means, that an adult homozygote carrier of the risk allele, has a 2–3 kg higher weight compared to non-risk allele [Frayling TM et al. 2007]. It is very interesting, that FTO is shown to influence the fat mass and was suggested to encode a 2-oxoglutarate-dependent nucleic acid demethylase, which is related to the regulation of food intake [Gerken T et al., 2007]. Also, it was the decreased lipolytic effect in adipocytes reported [Wahlen K et al., 2008]. The precise role of the FTO locus in obesity needs further examination, because it is still unclear whether FTO or the adjacent FTM gene is responsible for the effect. With reports of the discovery of FTO [Frayling TM et al. 2007; Dina C et al.,2007; Scuteri A et al.,2007] and the first, robust dichotomous trait associations [Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007], came the realization that the effect sizes detected would be smaller than anticipated and that successful analysis would need larger sample sizes than previously thought. This realization brought up large-scale international collaboration and meta-analyses of existing data. A strong connection was found between the SNPs located 188 kilobases (kb) downstream from the melanocortin 4 receptor gene (MC4R) and BMI through the first collaboration in obesity research (Tables 1 [Loos RJ et al.,2008]). In individuals of Indian Asian or European ancestry it was, in parallel found to be associated with WC (Table 1 [Chambers JC et al., 2008]). The risk variant has subsequently been linked to increased fat intake and energy [Qi L et al., 2008] and the higher BMI reported in children is consistent with early development of obesity caused byMC4R mutations [Farooqi IS et al., 2003]. Larger GWA meta-analysis, through the Genetic Investigation of Anthropometric Trait (GIANT) Consortia and deCODE (Table 1) followed and informed of 8 novel obesity loci, as well as supporting the MC4R and FTO associations [Willer CJ et al., 2009].Some of the likely causal genes in the associated regions are known to act or are highly expressed in the central nervous system (CNS), suggesting, as in rare monogenic forms of obesity, the role of CNS pathways in predisposition to overall obesity [Thorleifsson G et al.,2009]. Other forms of obesity (early onset, extreme obesity and/or morbid adult obesity) were examined in a few smaller GWAs [Meyre D et al.,2009; Cotsapas C et al., 2009; Scherag A et al.2010] and confirmed FTO, MC4R and TMEM18 BMI-associations. Also these studies reported four

novel associations. Cotsapas et al. [Cotsapas C et al., 2009] observed nominal evidence of association for 7 of the 13 loci previously identified to influence BMI [Willer CJ et al., 2009; Thorleifsson G et al.,2009; Meyre D et al.,2009]. The more severe forms of obesity represent the extreme, of the phenotypic spectrum rather than a distinct condition, this suggests that variants influencing BMI might be responsible, although, this needs further confirmation in appropriately powered studies.

A large-scale meta-analysis of 249 769 individuals supported recently 14 of these already identified obesity susceptibility loci and reported 18 novel loci associated with overall adiposity and BMI [Speliotes EK et al., 2010].Though the higher power of this study, new signals with smaller effect sizes and lower minor allele frequencies, compared to previously detected variants, were found. Further insights into the underlying biological mechanisms and pathways of overall obesity and weight regulation will be needed for the effective treatment and management of these traits but these results suggest that genome-wide association studies are only the first step in the identification of the causal variants that play a role [Speliotes EK et al., 2010].

3. Epigenetics and obesity



The superficial definition of epigenetics is the study of heritable mutations which affect gene function without changing the DNA sequence [Bird A. 2007]. The notion of epigenetic transmission is contentious and the maintenance of their marks through generations is poorly understood [Chong S et al., 2007]. Epigenetic tacks are specific for every tissue and include DNA methylation and histone modifications which mediate biological

processes such as imprinting. As many imprinted genes are regulators of gene expression controlling growth or growth factors, imprinting disorders often feature obesity as one of their clinical characteristics.

The expression of alleles according to their maternal or paternal origin is called genomic imprinting [Reik W et al., 1998]. It establishes a balance between the expression of the parental alleles influencing growth [Butler MG, 2009], resulting in counteracting growth effects of paternal and maternal genomes [Haig D, 1991]. In addition to growth, imprinted genes are also linked to differentiation, viability, development and metabolic functions [Smith FM et al., 2006].

There are two main clusters of genomic imprinting known in humans until today:

1. at region 11p15 containing several imprinted genes including IGF2, INS, KCNQ10T1 (LIT1) (paternally expressed) and H19, KCNQ1, CDKN1C, PHLDA2, KVLQT1 (maternally expressed) [Lee MP et al., 1999].
2. at 15q11–q12 a cluster of at least 7 imprinted genes, including;MKRN3, MAGEL2, NDN, SNURF–SNRPN (paternally expressed) and UBE3A, ATP10A (maternally expressed) [Schweizer J et al., 1999].

Numerous genetic events, like translocation, inversion, duplication, paternal disomy and hyper/hypo-methylation can occur due to failures in imprinting which result in obesity by altering expression of growth and cellular differentiation factors. For instance; the Prader–Willi syndrome (PWS) emerges by paternal deletion or uniparental disomy at 15q11–q13. It is a syndrome characterized by severe and sometimes life threatening form of obesity with an early onset caused by hyperphagia on basis of a dysfunction in the satiety centre [Shapira NA et al.2005]. In Albright hereditary osteodystrophy (AHO) a moderate obesity is to be found, due to disruption of imprinting at the GNAS gene (20q13.11 [Butler MG, 2009].

3. 1. Mediators of genomic imprinting

3. 1. 1. DNA methylation

DNA methylation plays an important regulatory role in eukaryotic genomes. Alterations in methylation can affect transcription and phenotypic variation [Murrell A et al., 2004], but the source of variation in DNA methylation itself remains poorly understood. Substantial evidence of inter-individual variation in DNA methylation exists with age [Rakyan

VK et al., 2010, Teschendorff AE et al., 2010], tissue [Eckhardt F et al., 2006; Gibbs JR et al. 2010], and species [Enard W et al.,2004]. In mammals, DNA methylation is mediated by DNA methyltransferases (DNMTs) that are responsible for de novo methylation and maintenance of methylation patterns during replication. Genes involved in the synthesis of methylation and in DNA demethylation can also affect methylation variation. For example, mutations in DNMT3L [El-Maarri O et al., 2009] and MTHFR [Friso S et al., 2005] associate with global DNA hypo-methylation in human blood. These changes occur at a genome-wide level and are distinct from genetic variants that impact DNA methylation variability in targeted genomic regions, for example, genetic polymorphisms associated with differential methylation in the *H19/IGF2* locus [Heijmans BT et al., 2007].

3. 1. 2. Histone modifications

Histone modification occure throughout the entire sequence, the unstructured N-termini of the histones are particulary highly modified. These modifications include acetylation, methylation, phosphorylation, ubiquitation and sumoylation.
The histone acetylation is most studied form of modification. For example, acetylation of the K14 and K9 lysines of the tail of histone H3 by histone acetyltransferase enzymes (HATs) is generally correlated with competence.
Different histone modifications are likely to function in different ways; acetylation at one position is likely to function differently than acetylation at another position. Also, multiple modifications may occur at the same time, and these modifications may work together to change the behavior of the nucleosome. The idea that multiple dynamic modifications regulate gene transcription in a systematic and reproducible way is called the histone code.
Also a feature of histone modifications is that it is both tissue specific and can vary with age (and developmental stage). Therefore, in order to place findings in an appropriate context, it is of major importance that evaluation of epigenetic factors be carried out on suitable tissues extracted at specified times [Feinberg AP, 2008; Feinberg AP, 2010].

4. Challenges

4. 1. Identifying novel loci

The combined effect of the loci explains only 2–3% of the inherited contribution to obesity risk. So despite the successes in susceptibility loci identification for obesity through the first wave of GWAs there is still a lot of work to be done.

4. 2. Collaborative studies to for larger GWA meta-analysis

The previously identified variants will elucidate, using the conditional analysis, whether some of the already identified loci contain more than one independent association with obesity, which would be a source of unexplored variation contributing to the missing heritability. Also, the next wave of GWA meta-analysis for obesity related traits incorporating >100 000 samples is finalized by the GIANT consortium. This increase in sample size should guaranty sufficient power to permit identification of common variants with even smaller effect sizes than hereto.

4. 3. Copy number variations

The first examples of copy number variants (CNVs) associated to obesity have been recently identified [Plagemann A et al., 2009] by two amendatory approaches. CNVs are hard to detect due to technical constrains. For example, the SNPs linked with obesity at the NEGR1 locus are in strong linkage disequilibrium (LD) with a nearby CNV [Willer CJ et al., 2009]. Recently by examining SNP genotype arrays that are applying novel CNV detecting algorithms and are enriched in CNV information, they reported on chromosome 16p11.2 was an associated with obesity [Walters RG et al., 2010; Bochukova EG et al.,2010]. Further approaches in larger sample sets should give a better understanding of total CNVs contribution to overall variation in obesity susceptibility.

4. 4. Correlation of genetic and epigenetic information

Genetic and epigenetic factors are not only co-existing but intimately intertlaced, as epigenetic marks and DNA modifications are the direct consequence of sequence-specific

interactions between DNA and proteins [Ptashne M et al., 2010]. The finalization of the human epigenome project will enlighten our understanding of the genetic and epigenetics underlying cellular homeostasis and the combination of this knowledge with the known environmental risks to obesity must be used so that it may eventually be applied and manipulated to reduce the risk for obesity.

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Serotonin system in the coordination of food intake and body weight

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) a biogenic amine is synthesized both in the enteric nervous system and the central nervous system (CNS). In the CNS, serotonin acts as a neurotransmitter and is released throughout most of the neuraxis. It is perhaps most commonly linked with the regulation of mood and anxiety, brain serotonin also coordinates numerous automatic, cognitive, and other functions to maintain homeostasis and ensure survival and reproduction.

2. Manipulation of endogenous serotonin and their effects on appetite

As we all know from many other research areas a lot of information can be gathered from the manipulation of perfect, harmonic systems and in this case we take a look at the endogenous serotonin synthesis, bioavailability, and metabolism. The manipulation provides important evidence for the role of endogenous serotonin in coordinating food intake and body weight. Together, these data illustrate the complex relationship between the level of brain serotonin signaling and food intake — when brain serotonin signaling is augmented, food intake is reduced, and vice versa.
Numerous genetic models of serotonin receptor deficiency have been generated as well as pharmacological targeting of serotonin receptors have been reported.

2. 1. Serotonin syntheses

P-chlorophenylalanine (PCPA) is an inhibitor of tryptophan hydroxylase activity and therefore inhibits serotonin synthesis. Intracerebroventricular (ICV) PCPA attendance in adult rats, specifically targeting brain serotonin synthesis, concludes in marked weight gain and hyperphagia for the duration of serotonin degradation [Breisch ST et al., 1976]. Though, serotonin is essential for normal development, and therefore tryptophan hy-

droxylase knockout mice show growth retardation and physiological dysfunction [Alenina N et al., 2009; Savelieva KV et al., 2008; Yadav VK et al., 2009].

2. 2. Serotonin bioavailability

The application of drugs can manipulate the bioavailability of endogenous serotonin which affect serotonin release or serotonin reuptake through the serotonin transporter. D-fenfluramine, which panders serotonin outlet from the intracellular compartment into the synapse through the serotonin transporter [Crespi D et al., 1997] and disables serotonin reuptake, produces hypophagia [Guy-Grand B, 1995; Halford JC et al., 2007]. Actually, D-fenfluramine was to a large extent prescribed to lose weight before its withdrawal due to adverse effects [Guy-Grand B, 1995]. Serotonin reuptake inhibitors, such as fluoxetine, sibutramine, and sertraline elevate extracellular serotonin levels and reduce appetite [Heal DJ et al., 1998; Heisler LK et al., 1997, 1999; Simansky KJ and Vaidya AH, 1990].

2. 3. Serotonin metabolism

The metabolism of serotonin can be inhibited with MAO-A inhibitors which are widely known as antidepressant drugs prescribed for the treatment of depression and not for appetite. These compounds increase extracellular serotonin levels and reduce food intake [Feldman JM, 1988].

2. 4. Serotonin receptors

The serotonin receptors are divided into 7 families on the bases of on evolutionary lineage, intracellular effectors and sequence homology, labeled as 5-HT1R to 5-HT7R [Nichols DE and Nichols CD, 2008]. Although some receptor families consist only of one single member (5-HT4R, 5-HT6R and 5-HT7R), the others contain several members: 5-HT1R includes 1A, 1B (also known as 1Dβ in humans), 1D (also known as 1Dα in humans), 1E and 1F subtypes; 5-HT2R includes 2A (formerly 5-HT2R), 2B, and 2C (formerly 1C); 5-HT3R includes 3A-E; 5-HT5R includes 5A and 5B.
Recent refined genetic studies now indicate that a primary mechanism through which serotonin influences appetite and body weight is via serotonin 2C receptor (5-HT2CR)

and serotonin 1B receptor (5-HT1BR). Therefore we now take a closer look at the different receptors and the complex system around serotonin.

2. 4. 1. 5-HT1R family

The inhibitory 5-HT1Rs allow feedback inhibition of serotonin neurons by serotonin. The 5-HT1AR subtype is located both postsynaptically and on the cell soma, whereas the 5-HT1BR is to a large extent expressed on terminals, but is also postsynaptically expressed. Application of 5-HT1AR agonists to dorsal and median raphe slices reduce serotonin release [Hopwood SE and Stamford JA, 2001], while the use of a 5-HT1BR agonist to the hippocampus, a target of 5-HT innervation, diminishes serotonin release [Hjorth S and Tao R, 1991]. In addition 5-HT1BRs are expressed on non-serotonergic terminals bocking the release of other neurotransmitters and neuropeptides [Barnes NM and Sharp T, 1999].
No changes in body weight or food intake were discovered in the initial observations of four different linages of 5-HT1AR knockout mice [Heisler LK et al., 1998; Parks CL et al., 1998; Ramboz S et al., 1998]. In 2008, a more recent research on one of the lines is inconsistent; one group showed no alterations in homecage feeding and body weight [Bechtholt AJ et al., 2008] whereas another found that the same line developed reduced food intake and fat pad mass [Yadav VK et al., 2009]. Locomotor activity was discoverd to be normal in the latter report. 5-HT1BR knockout mice displayed increased lengh and body weight, but not obesity (Bouwknecht et al., 2001). In behavioural satiety sequence analysis, 5-HT1BR knockout mice presented higher exploratory activity compared to wildtypes, but food intake was the same at all stages [Lee MD et al., 2004].
Also in a study examining the effect on appetite with the pharmacological targeting of the serotonin receptor, 5-HT1BR knockout mice showed attenuation of the anorectic response to D-fenfluramine [Lee MD et al., 2004; Lucas JJ et al., 1998]. However, 5-HT1BR knockout mice are resistant to the anorectic effects of 5-HT2CR agonists, too, an outcome that is not replicated by pretreatment with 5-HT1BR antagonists in wildtype mice [Clifton PG et al., 2003]. This supposes that an adaptive down regulation in 5-HT2CR activity or expression might emerge in the 5-HT1BR knockout mouse. In consensus with the complex relationship between serotonin signaling and intake of food, systemic treatment with 5-HT1AR agonists, which would reduce serotonin release through autoreceptor inhibition, specifically bring to light hyperphagia, without influenc-

ing grooming, drinking, rearing, or locomotion [Dourish CT et al., 1985] and 5-HT1AR antagonists diminishes palatable food intake [Moreau JL et al., 1992]. Completely different than 5-HT1AR agonists behaves 5-HT1BR agonists, which produce hypophagia and is diminished by 5-HT1BR antagonist treatment [Halford JC and Blundell JE, 1996; Lee MD and Simansky KJ, 1997]. These effects are probably due to heteroreceptor action on non-serotonin neurons. Indeed, potently and selectively reduced food intake can be attained by a discrete infusion of a 5-HT1BR agonist into the parabrachial nucleus of the pons, a serotonin target site [Lee MD et al., 1998]. 5-HT1BR agonism safes the structure of the behavioral satiety sequence, advancing the onset of resting [Halford JC and Blundell JE, 1996]. This study also noticed reduced rearing behavior following 5-HT1BR agonism, which supports the higher exploratory behavior shown in 5-HT1BR-deficient mice described previously.

2. 4. 2. 5-HT2R family

5-HT2CR knockout mice exhibited lifelong hyperphagia, with higher meal duration and frequency, and develop late-onset obesity [Nonogaki K et al., 1998; Tecott LH et al., 1995]. 5-HT2CR knockout mice also display a delayed behavioral satiety sequence with an intensified incidence of feeding and delayed onset of resting behavior [Vickers SP et al., 1999]. In addition to these findings the 5-HT2CR knockout mice also exhibited an increased locomotor activity in the homecage [Nonogaki K et al., 2003; Xu Y et al., 2008].

The 5-HT2CR is the only serotonin receptor for which genetic deficiency results in obesity and hyperphagia, which is suspected to plays an important role in the serotonergic coordination of food intake and body weight. 5-HT2CR knockout mice are in addition untouched by the anorectic effects of D-fenfluramine and mCPP [Tecott LH et al., 1995; Vickers SP et al., 1999]. The extent of the energy balance phenotype displayed by the 5-HT2CR knockout mouse is exactly the other way around by selective re-expression of the 5-HT2CRs exclusively in pro-opiomelanocortin (POMC) neurons [Xu Y et al., 2008]. No changes in food intake or bodyweight have been observed in 5-HT2AR or 5-HT2BR knockout mice [Nebigil CG et al., 2000; Weisstaub NV et al., 2006]. Be that as it may, a recent report suggests that selective bockage of 5-HT2BRs exclusively in POMC neurons decreases food intake and fat pad mass, but did not change locomotor activity [Yadav VK et al., 2009].

The significance of the 5-HT2CR in controlling food intake is supported by pharmacological studies as well. 5-HT2CR agonists reduce food intake in rodents, and these effects can be reversed by 5-HT2CR antagonists [Kennett GA and Curzon G, 1988; Kitchener SJ and Dourish CT, 1994; Martin RJ et al., 1998; Schreiber R and De Vry J, 2002]. The D-fenfluramine metabolite norfenfluramine is a full 5-HT2CR agonist [Curzon G et al., 1997], and D-fenfluramine hypophagia is diminished by 5-HT2CR antagonists [Vickers SP et al., 2001]. Corresponding to the 5-HT2CR knockout phenotype, pharmacological blockade of 5-HT2CRs reinforces food intake [Bonhaus DW et al., 1997]. 5-HT2CR agonism advances satiety in a manner consistent with a food preload [Kitchener SJ and Dourish CT, 1994]. In agreement with the hyperactivity of 5-HT2CR knockout mice, 5-HT2CR antagonism increases locomotor activity, while 5-HT2CR agonists diminish locomotor activity [Fletcher PJ et al., 2009; Martin JR et al., 1998]. Although 5-HT2AR agonists are linked to hypophagia, they also cause stereotypys [Fox MA et al., 2009], supposing that their effects may not be to food intake in general.

2. 4. 3. 5-HT3R family

For 5-HT3AR knockout mice there has been no food intake phenotype observed [Bhatnagar S et al., 2004]. However, pharmacological studies show that 5-HT3R is associated to aspect of ingestive behavior. The general 5-HT3R antagonist ondansetron, when it is administered to rats into the dorsal hindbrain, increases nutrient intake [Hayes MR and Covasa M, 2006]. But primarily the interest in 5-HT3R antagonists is to reduce vomiting and nausea in relationship with chemotherapy.

Table 1: Feeding and body weight phenotypes of mice with genetically altered serotonin-related genes

Genetic target	Feeding and body weight associated phenotypes	References
Htr1a−/−	No changes in body weight reported in four different lines of 5-HT1AR null mice. Additional analysis of one line by Bechtholt et al. reported higher intake of sucrose solution in females (potentially sex-hormone related), but no alterations in homecage feeding or body weight. In contrast, in the same 5-HT1AR null line, Yadav et al. found a smaller food intake and attenuated fat pad mass.	Bechtholt AJ et al., 2008; Yadav VK et al., 2009
Htr1b−/−	Mildly increased body weight and relative intensified in food intake and blunted re-	Bouwknecht JA et al., 2001; Lucas JJ et al.,

	sponses to anorectic serotonergic compounds	1998
Htr2a−/−	No changes in homecage feeding, novelty suppressed feeding or body weight reported.	Weisstaub NV et al., 2006
Htr2b−/−	None reported.	Nebigil CG et al., 2000
Htr2b/Pomc−/−	Mice lacking receptor expression in POMC neurons only, displayed reduced food intake and fat pad mass.	Yadav VK et al., 2009
Htr2c−/−	Hyperphagia throughout life and increased body weight gain from around 12 weeks. Attenuated responses to serotonergic anorectic compounds.	Tecott LH et al., 1995
Htr2c/Pomc	Selective re-expression of 5-HT2CR specifically on POMC neurons ameliorated the hyperphagic and obesity phenotype in 5-HT2CR knockout.	Xu Y et al., 2008
Htr2c−/−/Lepob/ob	Synergistic interaction followed by a hyperphagic phenotype greater than either mutation in isolation. Compound mutants decreased food intake to ob/ob levels by 5 months. Body weight of double ob/2C null was comparable to ob/ob mice.	Wade JM et al., 2008
Htr3a−/−	No observed differences in body weight or food intake.	Bhatnagar S et al., 2004
Htr4−/−	Modestly reduced weight gain in homecage environment, despite normal food intake. Diminished restraint stress-induced hypophagia.	Compan V et al., 2004; Jean et al., 2007
Htr5a−/−	Normal body weight. No data on food intake reported	Grailhe R et al., 1999
Htr6−/−	Normal chow intake and body weight. On high fat diet, 5-HT6R knockouts are hypophagic and resistant to obesity.	Bonasera SJ et al., 2006
Htr7−/−	Normal body weight. No data on food intake reported.	Hedlund PB et al., 2003

2. 4. 4. 5-HT4R

In 5-HT4R knockout mice there have been no abnormalities in basal food intake displayed, but these mice exhibited diminished stress-induced hypophagia [Compan V et al., 2004]. A 5-HT4R agonist infused into the nucleus accumbens reduces food intake,

while application of a 5-HT4R antagonist, as well as intraaccumbal 5-HT4R siRNA-mediated knockdown, produce hyperphagia [Jean A et al., 2007].

2. 4. 5. 5-HT5R family

There have been no data on food intake reported. The 5-HT5AR knockout mice are characterized to exhibit completely normal body weight [Grailhe R et al., 1999].

2. 4. 6. 5-HT6R

Although 5-HT6R knockout mice display a normal intake of regular chow [Bonasera SJ et al., 2006], these mice are hypophagic and resistant to diet-induced obesity even if they are exposed to a high fat diet [Frassetto A et al., 2008]. Likewise, 5-HT6R antagonists diminishes food intake [Heal DJ et al., 2008; Perez-Garcia G and Meneses A, 2005; Woolley MI et al., 2001] and ICV administration of a 5-HT6R antisense oligonucleotide reduces food intake [Woolley MI et al., 2001].

2. 4. 7. 5-HT7R

5-HT7R knockout mice display normal body weight [Hedlund PB et al., 2003] and no data linked to food intake in this line of mice has been found.

3. Other reward systems and regulation

3. 1. Dopamine

Stanley, Wynne, McGowan, and Bloom [Stanley S et al., 2005] have observed that the dopaminergic system is crucial to reward-induced feeding behavior. Mice that lack dopamine have fatal hypophagia.
Terasawa and Fernandez [Terasawa E & Fernandez DL, 2001] suggest that dopamine affects to great extent also pubertal development through its influence on the release of luteinizing hormone-releasing hormone. Their recent animal studies revealed that dopamine levels display a particular increase right before or around the time of puberty. Studies of rats and nonhuman primates suggest that dopaminergic input to the prefrontal cortex increases during adolescence, peaking at levels higher than those seen earlier or later in development [Spear LP, 2000].

3.2. Norepinephrine

A bidirectional regulation of appetite is influenced by the noradrenergic system. Activation of α1- and β2-adrenergic receptors inhibits appetite, and norepinephrine reuptake inhibitors that act on these receptors (e.g., phentermine) lower the food intake.
In contrast, the α2-adrenergic receptors activate an increased food intake [Stanley S et al., 2005].

3. 3. Opioids

An important role in the reward value of food plays the endogenous opioids, which include endorphins and enkephalins. For mice lacking enkephalin or endorphin the food loses its reinforcement potential [Stanley S et al., 2005]. Opiate antagonists in humans reduce food palatability without altering subjective hunger [Stanley S et al., 2005]. This means that even though opioids are not directly responsible for hunger, they affect the hedonistic pathways that make food consumption tempting and pleasurable.

3. 4. Other possible factors

The following factors of biological and psychological processes are not intended to be a complete list of moderators, but a short glance to other systems and processes that could maybe influence weight and appetite.

Increased levels of proinflammatory cytokines, proteins that regulate immunity and inflammation, may influence weight and appetite symptoms [Carney RM et al., 2002; Raison CL et al., 2006]. In concert with leptin, proinflammatory cytokines influence the central nervous system to reduce food intake [Dixit et al., 2004].

In addition, research assumes a possible role for proinflammatory cytokines in the timing of pubertal development viamediational effects on leptin [Quinton ND et al., 1999; Sarraf P et al., 1997].

Exogenous cytokine application, either centrally or peripherally, influences mood and eating behavior [Konsman JP et al., 2002; Markowitz S et al., 2008].

Another area of research suggests an important role for early life events. Neural systems are quite plastic and easy to manipulate in early development, such that early deprivation, abuse, or trauma can lead to lasting and varied impairments through epigenetics, alterations in the expression of genes via DNA without changes in coding sequence [Holmes A et al., 2005]. Pace et al. [Pace TWW et al. 2006] showed that among a group of men with major depression, those with higher levels of stress in their early life time displayed greater inflammatory responses to psychosocial stressors as adults.

4. Hormones

4. 1. Corticotropin-releasing hormone (CRH)

CRH is a hormone associated to the body's stress response, indirectly stimulating the output of glucocorticoids. Animal studies show that an over-expression of CRH leads to a higher food intake and weight gain (Bornstein SR et al., 2006). The activity of CRH seems to change during life time and especially in adolescence. Research assumes that CRH cells may respond differently to acute and chronic stress in prepubertal versus postpubertal animals, with a larger number of CRH cells activated during the stress response of prepubertal animals [Romeo RD & McEwen BS, 2006]. It is very interesting that a sex difference in cortisol response to CRH seems to display in adolescence, with girls showing higher cortisol levels to CRH across pubertal development and boys showing relatively stable cortisol responses across pubertal time [Stroud LR et al., 2004]. CRH hypersecretion appears to mediate several depressive symptoms, including appetite disturbance, sleep disturbance, reduced libido, and psychomotor disruptions [Arborelius L et al., 1999].

4. 2. Glucocorticoids

A part of the stress response are the glucocorticoids, such as cortisol, which are steroidal hormones secreted by the adrenal cortex. Exogenous administration of glucocorticoids is generally followed by metabolic changes typical of obesity [Bornstein SR et al., 2006].
In addition to its role as a stress hormone and regulator of the hypothalamic-pituitary-adrenal axis, another study observed that the treatment with D-fenfluramine elevates Crh mRNA levels in the paraventricular hypothalamic nucleus (PVH), and 5-HT2CR null mice display diminished PVH Crh mRNA [Heisler LK et al., 2007].
Also, PVH CRH neurons are activated by systemic application of D-fenfluramine and serotonin receptor agonists [Bovetto S et al., 1996; Javed A et al., 1999].
Pretreatment with an anti-CRH antibody inactivates the anorectic effect of some doses of centrally injected serotonin or DL-fenfluramine [Le Feuvre RA et al., 1991].
Even though PVH CRH neurons were given direct doses of serotonin [Liposits Z et al., 1987], the activation of CRH neurons noticed after serotonin level elevation that the

melanocortin system may be at least in some parts a secondary effect of activation by serotonin. PVH CRH neurons express melanocortin 4 receptors (MC4Rs), and are extremely fast activated by melanocortin receptor agonists [Lu XY et al., 2003]. Moreover, pharmacological blockade of CRH receptors diminishes the anorectic effect of a melanocortin receptor agonist [Lu XY et al., 2003].
So the conclusion we could draw from those studies is that serotonin's effects on CRH activity may be direct, but may also be indirect through the effects of the melanocortin system.

4. 3. Specific gonadal hormones

Gonadal hormones (for instance estradiol and testosterone) have an enormous effect on body composition and weight. After the first six months of life, levels of gonadal hormones are low until puberty, at which time the levels are reinforced dramatically [Terasawa E & Fernandez DL, 2001]. Animal studies have shown that testosterone reduces adiposity, or fatty tissue, whereas progestin causes elevated in body weight [Wade GN & Gray JM, 1979]. These typical different effects of gonadal hormones for males and females are responsible for some of the common sex differences in relative composition of body weight gained during pubertal development (with males gaining relatively more muscle and less fat than females).
The sudden rise of pubertal hormones is also linked to alterations in affect and risk for psychopathology. The surge of gonadal hormones changes the sensitivity of neurotransmitter systems in ways that influence mood [Steiner M et al., 2003]. The surges of hormones sets the genders apart in ways that may make females more vulnerable to negative affect and depression. [Susman EJ et al., 1987].

4. 4. Leptin

Leptins a peptide hormone that was already mentioned in combination with inflammatory cytokines but plays also on its own an important role in weight regulation and adiposity. A person's total fat mass is proportional to its circulating leptin levels, and administration of leptin to animals is followed by a marked reduction in food consumption and weight loss [Neary NM et al., 2004]. Longitudinal research has observed that during adolescence, levels of leptin elevate in females and reduce in males [Ahmed ML et al.,

1999]. This sex difference in leptin levels during adolescence is related to the fact that both sexes gain weight, but girls gain proportionally more fat mass than boys [Ahmed ML et al., 1999].

Licinio and Wong [Licinio J &Wong ML, 1999] assume that leptin likely influences symptoms of appetite and weight dysregulation also in depression. It is in fact very interesting that different leptin abnormalities may be responsible for depression-related weight loss or weight gain. This theory is supported by Kraus et al. [Kraus T et al. 2001] who demonstrated that individuals suffering from depression had normal BMIs, but diminished leptin levels when compared to healthy controls. Completely different evidence found Gecici et al., [Gecici O et al., 2005] in their study, individuals with atypical depression (characterized by hyperphagia, weight gain, hypersomnia) had higher serum leptin levels than the healthy controls.

5. Hypothalamic controls

5. 1. Hypothalamus

Hypothalamic dysfunction is the perhaps best studied genetic cause of obesity and is widely known to be a control of body weight [Swaab DF, 1997]. The different parts of the hypothalamus regulatory center for both elevated and reduced weight and appetite, with lesions in the ventromedial hypothalamus leading to higher food intake and weight gain, and lesions in the lateral hypothalamus leading to undereating and weight loss [Rosenbaum M & Leibel RL, 1998]. It is due to this research that we consider the ventromedial hypothalamus as a satiety center and the lateral hypothalamus as a feeding center.
The hypothalamus is the center of production of orexins and oxytocin.

5. 1. 1. Orexins

Orexins (=hypocretins) are orexigenic neuropeptides and are produced by neurons in the lateral hypothalamus [Nambu T et al., 1999; Sakurai T et al., 1998]. Orexins is an important factor in the coordination of arousal with food-seeking behaviour [Saper CB, 2000]. Dense serotonin terminals surround orexin neurons and hyperpolarize following to serotonin application [Muraki Y et al., 2004].

5. 1. 2. Oxytocin

Oxytocin which is established by neurons of the PVH and supraoptic nucleus of the hypocampus plays a role in uterine contractions, maternal and social bonding and lactating. Since injection of oxytocin into the dorsal motor nucleus of the vagus reduces gastric motility, it is suggested that oxytocin neurons may influence food intake via projections to the dorsal vagal complex [Rogers RC and Hermann GE, 1987]. Oxytocin neurons are activated, and oxytocin secretion is reinforced, by D-fenfluramine and serotonin receptor agonists [Jorgensen H et al., 2003; Osei-Owusu P et al., 2005; Van de Kar LD et al., 1995, 2001; Zhang Y et al., 2002].
Like the already discussed CRH, the involvement of oxytocin in the serotonergic system of food intake may be another factor aside a lot of factors to melanocortin system activation.

5.2. Neuropeptide Y

Neuropeptide Y (NPY) is a peptide released from the arcuate nucleus that is centrally involved in hunger and meal initiation [Jequier E & Tappy L, 1999]. Nutrient absorption results in a feedback inhibition of NPY, leading to reduced hunger. NPY exerts powerful effects on appetite and likely interacts with several other peptides and feedback loops [Jequier E & Tappy L, 1999].
NPY is co-expressed with the melanocortin receptor antagonist agouti-related peptide (AgRP) [Broberger C et al., 1998; Hahn TM et al., 1998]. These neurons receive serotonin inputs [Guy J et al., 1988; Heisler LK et al., 2006] and are hyperpolarized by 5-HT1BR agonists [Heisler LK et al., 2006]. Levels of NPY, and its mRNA, are decreased by pharmacological serotonin reinforcement [Choi S et al., 2006; Dryden et al., 1996]. Additionally, feeding induced by NPY administration is diminished by D-fenfluramine [Bendotti C et al., 1987; Grignaschi G et al., 1995]. The blockage of orexigenic NPY/AgRP neurons by 5-HT1BR action, directly linked with the activation of opposing anorexigenic POMC neurons by 5-HT2CR action, that assumes that these receptors support each other's effects on at least one pathway.

5. 3. Melanocortins

Melanocortins are anorectic hypothalamic neuropeptides which are prominently involved in control of body weight and appetite. In both rodents and humans a mutation in the genes encoding the endogenous melanocortin agonist precursor POMC and the MC4R result in heavy hyperphagia and obesity [Challis BG et al., 2004; Farooqi IS et al., 2000; Huszar D et al., 1997; Krude H et al., 1998; Yaswen L et al., 1999; Yeo GS et al., 1998]. Serotonin reinforcment with D-fenfluramine, a 5-HT2C/1BR agonist, activates POMC-expressing neurons in the hypothalamic arcuate nucleus, which is a subpopulation that express 5-HT2CRs [Heisler LK et al., 2002; Lam DD et al., 2008].
Also interesting is that serotonin and 5-HT1BR agonists blockes neurons expressing the endogenous melanocortin receptor antagonist AgRP, a subpopulation of which express 5-HT1BRs [Heisler LK et al., 2006]. A study in 2008 demonstrated that selective 5-

HT2CR expression only on POMC neurons is enought to normalize the hyperphagia, obesity, and attenuated the answer to anorectic serotonergic drugs displayed by 5-HT2CR null mice [Xu Y et al., 2008].

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Theory of a Meta-analysis

1. Introduction

It is exceptionally hard to stay on top of every subject in this fluently and fast changing world. In the 17 century Gottfried Wilhelm Leibniz was maybe the last universal scientist for whom it was possible to keep track of and know everything.
Since that time which also marks the beginning of modern science, the production of scientific knowledge has grown exponentially. This can be gathered from the explosive increase of journals. In 1750 there were roughly ten scientific magazines. This number increased with great exactness ten times every 50 years till the end of twentieth century. For today`s scientists, which are facing more and more published investigations, it is hardly possible to get and keep an overview of all research works even in a clearly defined subject matter. Furthermore, in some research areas there are inconsistent and even sometimes opposing outcomes.
This development illustrates the increased need for a possibility to condense information and assess scientific outcomes, which led to the development of different methods to summarize outcomes. In addition to the traditional form of review, quantitative results combining studies, called meta-analysis came up during the 1970ies.

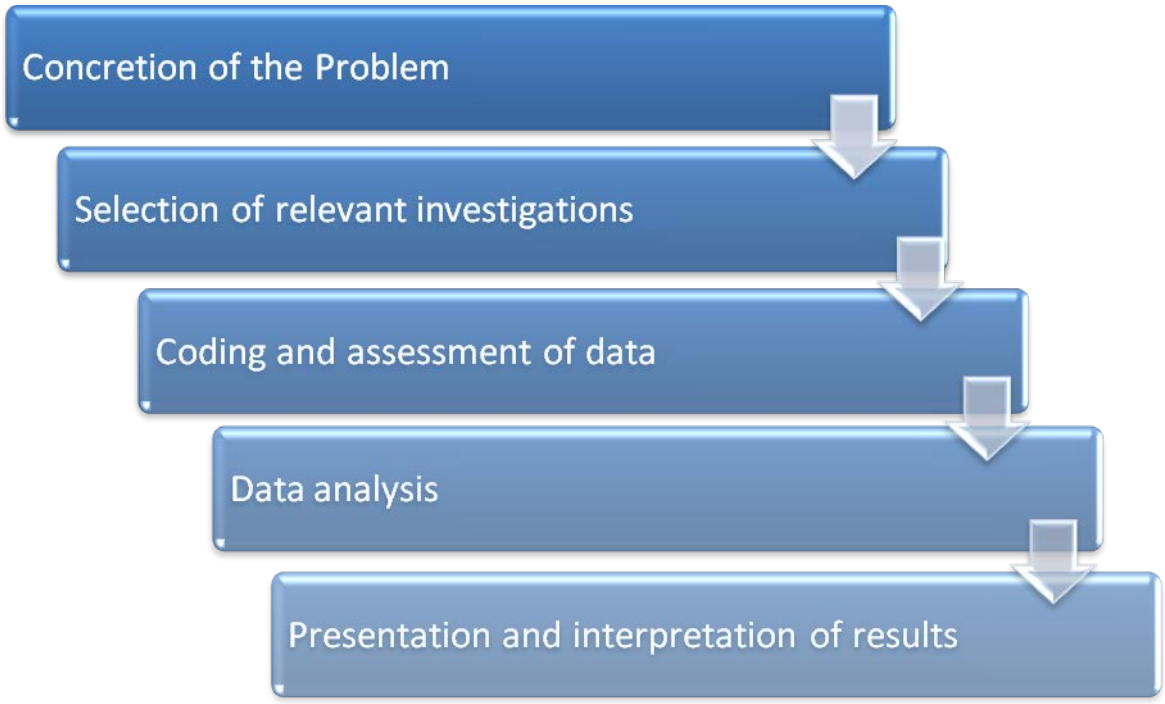
2. History and essence of meta-analysis

The first known quantitative result combining study, which we recognize nowadays as meta-analysis, was undertaken by the mathematician Karl Person in 1904 [Pearson, K, 1904]. At that time the success of the typhus vaccination was not yet proven, particularly because the studies were conducted with a far too small sample size and led to differing outcomes.
Since the 1950ies the first quantitative result integrations were used for questions concerning psychology and education science by Glass. The birth, of what we now call meta-analysis is dated in the mid 70ties, as Gene V. Glass introduced his method of quantitative result integration in a talk at the annual conference of the American Education Research Association [Glass, GV et al. 1981]. After that date there was an uptake in the

number of studies combining quantitative results of previously conducted studies and also in the critical methodological reflection about the meta analytic method.
What is really the essence of a meta-analysis? When Glass introduced the notion meta-analysis, he differentiated the method as a kind of third-analysis not a primary or secondary analysis. ”Primary analysis is the original analysis of data in a research study.(…) Secondary analysis is the re-analysis of data for the purpose of answering the original research question with better statistical techniques, or answering new questions with old data. (…) meta-analysis refers to the analysis of analysis (…) the statistical analysis of large collection of analytical results from individual studies for the purpose of integrating the findings” [Glass, GV, 1976]

3. Structure of meta-analysis

The carrying out of meta-analysis is simple in comparison with other known investigations of empiric research, like the interviewing of people, just that a meta-analysis is a study were the examination results represent the objects of the investigation. The investigation objects are coded, with regard to the interviewed properties and the established results are assessed by a statistic method chosen by the researcher [Cooper, H & Hedges LV, 1994b; Cooper, H, 1984; Durlak, JA & Lipsey MW, 1991].
The following graph shows the prototype structure of a Meta-analysis:



3. 1. Concretion of the Problem

Like every other examination, a meta-analysis starts with the concretization of the problem, the main research question. The formulation and the rough specification of investigated variables should be clear.

3. 2. Selection of relevant investigations

The collection of relevant investigations is the data survey of the meta-analysis. All the studies need to be defined with regard to the examined variables, i.e. it must be decided, which relationship of variables in the study will be investigated, so the individual study is chosen for the meta-analysis.
Also other selection criteria can be a applied, like the type of publication (journals, congress speeches and working papers), the time frame of relevant investigations or the cultural and especially the language of the examination [Lipsey, MW & Wilson, DT, 2001]
Only extended research and data collection guaranties that all important works will possibly be recognized and consequently no systematic bias will appear. Today electronic databases are mainly used for the collection of data. To guarantee even more information the references of the collected works can be reviewed or scientist can be contacted for further information.

3. 3. Coding and assessment of data

The coding and calculation of the found data follow as next steps. Here the usefulness of the individual studies for the meta-analysis is finally checked and in particular all informations which are important for the meta-analytical calculation, are coded.

3. 4. Data analysis

The data analysis involves two steps: first, the integration of individual results, and second the examination of the variation of the individual results in comparison.
In principle, next to the effect strength measurement for investigation results, there are also the integration of the level of significance or the simple possibility of counting out the significant and non-significant results.

3. 5.Presentation and interpretation of results

With the presentation and interpretation of results the methodological steps should be described and the results should be pointed out and be summarized. The application for theory and practice should be mentioned and especially the possible scientific gaps, as well as the further research possibilities should be discussed [Halvorsen, KT, 1994].
The representations of the studies with the relevant properties, as well as the results themselves appear mainly in the form of tables. But it is also possible and in many cases helpful to use a graphic description [Light, RJ et al. 1994].

4. Problems of meta-analysis and how to avoid them

The meaning and value of meta-analysis has been continually and intensively discussed in particular during the 1970ies. After the first phase of propagation, the meta-analysis was severely criticized. This phase again was followed by a consolidation, establishment and frame setting of the method [Drinkmann, A, 1990].
Now what exactly under current status of methodological development pleads for the use of meta-analysis, and what against?
Considering the aim of meta-analysis, which again is the combination of study results that address a set of related research hypotheses, the meta-analysis has the following clear advantages over a classical review of literature [Beelmann, A & Bliesener T, 1994; Drinkmann, A, 1990; Glass, GV et al. 1981; Hunter, JE & Schmidt FL, 1990; Plath, I, 1992; Wolf, FM, 1994]:

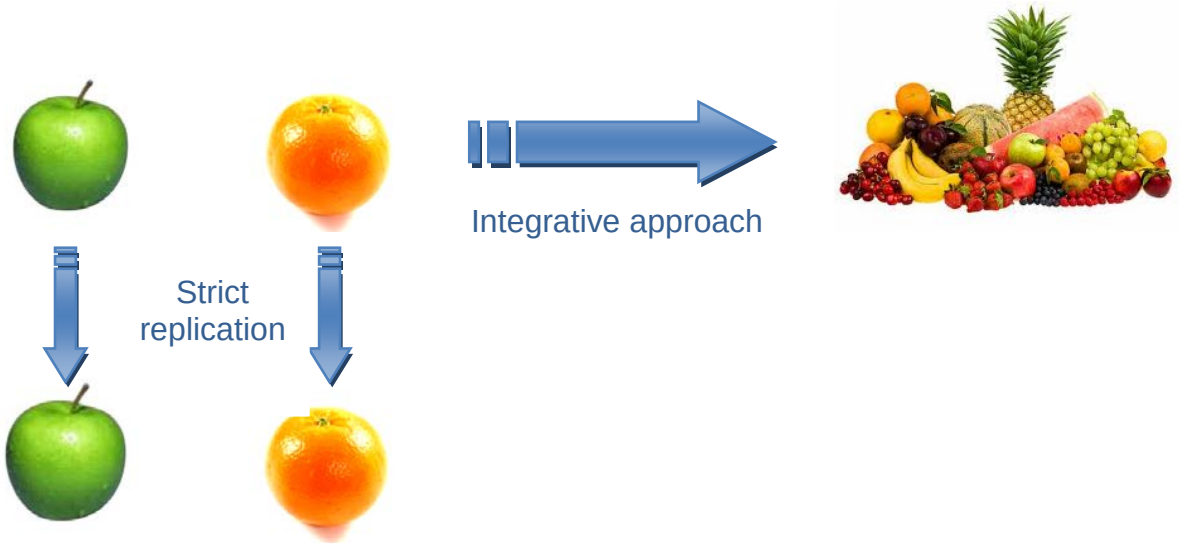
- Shows if the results are more varied than what is expected from the sample diversity
- Derivation and statistical testing of overall factors/ effect size parameters in related studies
- Generalization to the population of studies
- Ability to control the variation between study
- Higher statistical power to detect an effect than in n=1 sized study sample
- Including moderators to explain variation

- Dealing with information overload: the high number of articles published each year
- It combines several studies and will therefore be less influenced by local findings than single studies will be
- Makes it possible to show if a publication bias exists

Almost every literature source concerning meta-analysis mentions the following 4 points as the most important critics and counter-arguments.
Today these problems are all to a great extent discussed and these following paragraphs should just refer to the awareness of existing problems and how I handled this in the conducted meta-analysis.

4. 1. Uniformity problem

Meta-analysis combines many study results of a researched problem. These studies can be very different in themselves: the method used, the characteristics of populations in the analysis considering sample size or analyzing method. Such differences create problems of comparability. Two ways and scientific positions exist to deal with the identification and resolution of this problem [Hunter, JE, 2001]:



One way is a very strict approach, which only accepts perfect replications to be meta-analyzable. This means that only studies using the same variable relations with the same analyzing method are examined [Lipsey, MW & Wilson, DT, 2001]. This approach is mainly used in natural sciences.

Another very provocatively opposing way is the claim for integration by Glass. Where the meta-analysis is not a mere replication study, concerned with the analysis of variances, but a combination of all studies, which have the same question in mind, and which the researcher considers to be essential and methodological homogeneous. The depiction of Smith et al.`s frequently quoted statement fits this well: “Indeed the approach does mix apples and oranges, as one necessarily would do in studying fruit.”

4. 2. Publication bias

The critic of the publication bias relates to the selection mechanism in the investigation and publication process, where the significant results are promoted and the non-significant are filed unpublished in a drawer. So next to the published works there is probably a certain grey-number of examinations [Wolf, FM, 1994].

One very simple possibility to examine the existence of a publication bias is the funnel-graphs.

Funnel plots, introduced by Light and Pillemer in 1984 [Light, RJ & Pillemer, DB, 1984] and discussed in detail by Egger and colleagues [Egger M et al., 1997; Sterne JAC & Egger M, 2001] are useful adjuncts to meta-analyses. A funnel plot is a scatter plot of treatment effect against a measure of study size. It is used primarily as a visual aid to detecting bias or systematic heterogeneity. A symmetric inverted funnel shape arises from a ‘well-behaved’ data set, in which publication bias is unlikely. An asymmetric funnel indicates a relationship between treatment effect and study size. This suggests the possibility of either publication bias or a systematic difference between smaller and larger studies (‘small study effects’). Asymmetry can also arise from use of an inappropriate effect measure. Whatever the cause, an asymmetric funnel plot leads to doubts over the appropriateness of a simple meta-analysis and suggests that there needs to be investigation of possible causes.

A variety of choices of measures of ‘study size’ is available, including total sample size, standard error of the treatment effect, and inverse variance of the treatment effect (weight). Sterne and Egger have compared these with others, and conclude that the standard error is to be recommended [Sterne JAC & Egger M, 2001]. When the standard error is used, straight lines may be drawn to define a region within which 95% of

points might lie in the absence of both heterogeneity and publication bias [Sterne JAC & Egger M, 2001].

In common with confidence interval plots, funnel plots are conventionally drawn with the treatment effect measure on the horizontal axis, so that study size appears on the vertical axis, breaking with the general rule. Since funnel plots are principally visual aids for detecting asymmetry along the treatment effect axis, this makes them considerably easier to interpret.

Of course there is also a mathematical or analytical approach to check for publication bias.

4. 3. Integration of studies of different quality

= Garbage in, Garbage out

This critical point relates to the differences concerning the methological quality of the studies used. The problem is mainly founded on the assumption of the relation between methological quality and result of a study: the higher the methological quality, the stronger are the results. This problem could simply be resolved by excluding studies of inferior quality. But the difference in study quality can also be recognized in the integration of the effect size, as a kind of weight factor. Or one refers to the study quality, as a moderation variable, to explain the heterogeneity of the integrated data.
An a priori exclusion of studies with inferior quality, which is proposed for a very strict approach of a meta-analysis, has the down side of less information, because the totality of the possibly included data gets smaller.

4. 4. Non-independent effects

Finally, with the integration of research results, the problem of dependence of results (non-independent effects or multiple effect size) is to be taken into consideration. This last criticical point of the meta-analysis relates to the fact, that several relevant effects of one study, which were identified with the same investigation objects, are statistically not independent from each other and when interpreted these dependent effects can result in a distorted integration result.

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Meta-analysis: Obesity and the HTR2C polymorphism (-759 C/T)

1. Introduction:

Serotonin (5-hydroxytryptamine, 5-HT) one of the most important neurotransmitters is regulating energy balance and is synthesized from the amino acid tryptophan. 5-HT is acting in many complex events of human physiology, from the gastrointestinal and cardiovascular systems to centrally mediated activities such as mood, sleep, appetite, glucose homeostasis, temperature, sexual behavior, learning, and memory. The actions of serotonin are conducted through seven families of 5-HT-receptor subtypes that have been studied with different intensity until now.

In the meta-analysis I took a closer look at the 5-HT2C receptor, especially the SNP -759C/T and its association to obesity. The results of the meta-analysis were compared to a large population-based cohort association study [Vimaleswaran KS et al., 2010] and another meta-analysis [De Luca V et al. 2007] which was conducted using pharmacological induced weight gain and not general obese subjects.

5-HT2C receptor couples to multiple intracellular signaling pathways, is expressed in the hypothalamus and paraventricular nucleus [Miller KJ et al., 2005] and its gene is located at the chromosome Xq24 [Stam NJ et al., 1994].

The site of the -759C/T polymorphism contains regulatory and putative transcription factor binding regions [Shih JC et al., 1996] that regulate levels of the receptor protein, which could potentially change the neuronal regulation of many physiological processes including appetite. The -759T allele is functional and has been associated with greater promoter activity than the wild-type -759C allele in Yuan X et al. [Yuan X et al., 2000], a study we also have included in our meta-analysis. The 5-HT2C receptor is of particular interest in the weight gain liability as serotonin plays an important role in the energy balance and appetite regulation centers in the central nervous system (CNS) located in a region where lesions result in obesity [Parkinson WL et al., 1990].

2. Methode

2. 1. Concretion of the Problem

Genetic association studies of the -759C/T (SNP rs 3813929) polymorphism in adults assessed for obesity was included. Investigations reporting of any ethnic origin were included.

2. 2. Selection of relevant investigations

A literature search was performed in July 2011 using the PubMed online search engine. This database was searched from the year 2000 using the search terms “5-HT2C” and “HTR2C”. Additional publications were retrieved by reviewing the references of selected studies.

2. 3. Coding and assessment of data

For each study, the following data were extracted using standard forms: author, year of publication, sample ethnicity, case and control sample size, allele frequency, mean age, sex ratio. Ethnicity was coded as Caucasian and Asian.

2. 4. Data analysis

I used SPSS Statistics 17.0 for the descriptive statistics, like means, summes and standard deviation. Then I used a Microsoft Excel sheet by Rob Herbert to calculate the Odds and Confidence Intervals. The method used to calculate a confidence interval for the difference between two proportions is the Newcombe-Wilson method without continuity correction. The confidence limits for the number needed to treat are the inverse of the limits for the absolute risk reduction. Confidence intervals and odds ratios are calculated using the methods described by Armitage and Berry [Armitage P and Berry G 1994].

2. 5.Presentation and interpretation of results

Literature search identified a total of 127 papers, of which 123 had to be excluded because of various reasons, for example:

- conducted in children
- no healthy control populations included
- incomplete data about polymorphism
- subjects were not obese
- animal study
- Weight gain induced by pharmaceutics

The remaining four studies, all association studies, described a total of 3396 participants, divided in an obese of 1301 and a control group of 1850 participants.
This resulted in a 2662 wild typ subjects and 489 being homozygote for the -759 C/T SNP.

Table 1: Overview of complete numbers of participants

	Obese	**Control**
N	**1301**	**1850**
Genotyp Ho	**198**	**291**
Genotyp Wt	**1103**	**1559**

Table 2: The following studies were included in the analysis and in the following comparison:

Study	*Year*	*n*	*Ancestry*
Yuan X et al.	2000	589	Asian
Iordanidou M et al.	2008	289	Caucasian
Kring SII et al.	2009	s. 46: 1557 s.49: 485	Caucasian Caucasian
Bah J.et al.	2010	231	Caucasian
Compaired with:			
Vimaleswaran KS et al. ***Association study in a large population-based cohort***	2010	4978	Caucasian
De Luca V et al. ***Meta-analysis: Antipsychotic induced weight gain***	2007	588	European, Asian, Hispanic and African-American

The first study by **Yuan et al.** consisted of 589 men aged 51 ± 10 years having body mass index (BMI) of 25.2 ± 4.0 kg/m^2 recruited from people who attended the Kumamoto Red Cross Health Care Centre and patients treated at the Kurume University Hospital. The subjects included 123 obese (BMI ≥ 28) and 466 non-obese (BMI < 28) men.
The second group of participants from **Iordanidou M et al.** included 107 (49 males and 58 females) classified as obese [body mass index (BMI) ≥ 30 kg/m2] and 182 (113 males and 69 females) classified as nonobese (BMI<30 kg/m2). All patients were of Caucasian (Greek) ethnic origin.
In the third study by **Kring SII et al.** a population of obese men (n = 726, BMI ≥ 31.0 kg/m^2) and a randomly selected normal weight group (n = 831) were re-examined at two surveys at mean ages 46 and 49 years (S-46, S-49). In the first survey there were 1557

(726 obese and 831 randomly selected) participants and in the re-examination there were 485 (209 obese and 276 randomly selected) assessed.

The last study by **Bah J et al.** comprises 231 male (normal/obese) participants from Sweden which were included in my analysis.

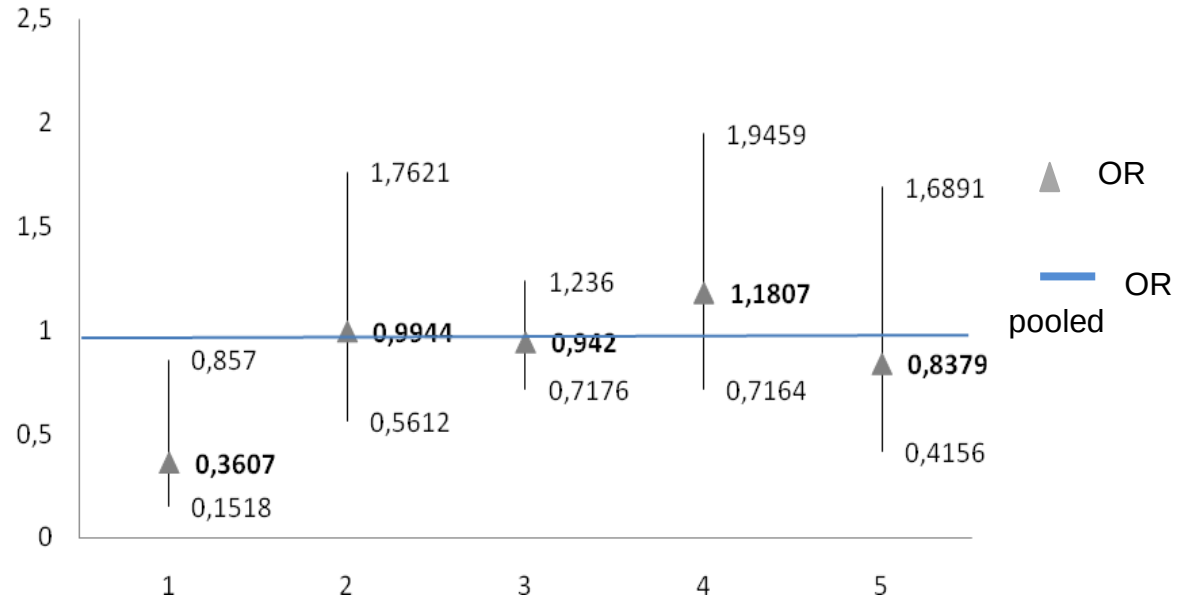
All of the studies investigated the association of the single nucleotide polymorphisms (SNPs) of the HTR2C gene with obesity. For the genetic analysis **Yuan X et al., Bah J.et al.** and **Iordanidou M et al.** used a slightly different polymerase chain reaction restriction fragment. And **Kring SII et al.** used a Taqman allelic discrimination.

All the four studies taken together resulted in an odds ratio of 0,9617 (95% CI: 0,7903 – 1,1703) for the effect of T variant to be associated with lower weight (Table 3).

Table 3: Odds ratio and confidence intervalls of all four studies and participants taken toghther

Relative Risk:	**0,9675**	**CI:**	**0,8195**	**to**	**1,1423**
Odds ratio:	**0,9617**	**CI:**	**0,7903**	**to**	**1,1703**

Figure 1:



Yuan et al.	Iordanidou M et al.	Kring SII et al.	Kring SII et al.	Bah J.et al.

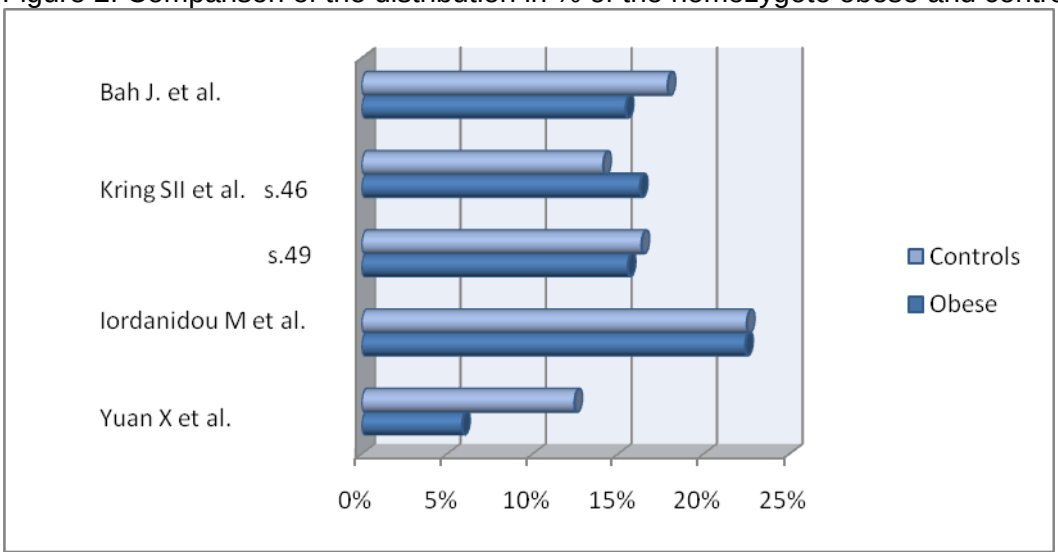
The vertical forest plot of studies is assessing the effect of homozygote or T variant with higher weight: overall genotype effect for dichotomous outcomes (random-effects model). Odds ratio (OR)>1 in favor T effect higher weight; OR<1 against T higher weight. The blue line presents the pooled OR of the four studies.
The forest plot clearly shows that the studies by **Iordanidou M et al., Kring SII et al. and Bah J.et al.** are not significant for an association with a higher weight. Also the pooled OR gives no indication for a signifcans. The only runaway is the study by **Yuan et al.** which associates the homocygote / T variant with a lower body weight.

Table 4: Genotyped distribution in (%) for the examined SNP in obese and controls participants in all studies

Survey	**Total sample size**	**Obese**					**Contr.**				
			Genotyp					**Genotyp**			
			Wt n	**(%)**	**Ho n**	**(%)**		**Wt n**	**(%)**	**Ho n**	**(%)**
Yuan X et al.	**589**	**123**	**117**	**(95,122)**	**6**	**(5,878)**	**466**	**408**	**(87,553)**	**58**	**(12,447)**
Iordanidou M et al.	**289**	**107**	**83**	**(77,570)**	**24**	**(22,430)**	**182**	**141**	**(77,473)**	**41**	**(22,527)**
Kring SII et al.	**1557**	**726**	**613**	**(84,435)**	**113**	**(15,565)**	**831**	**695**	**(83,634)**	**136**	**(16,366)**
	485	**209**	**175**	**(83,732)**	**34**	**(16,268)**	**276**	**237**	**(85,870)**	**39**	**(14,130)**
Bah J.et al.	**231**	**136**	**115**	**(84,558)**	**21**	**(15,442)**	**95**	**78**	**(82,105)**	**17**	**(17,895)**

To assess the association of the -759 C/T polymorphism with obesity, I additionally worked out the distribution in % of the homozygote (T variant) obese participants and control participants which is in both Table 4 and Figure 2 displayed. The homozygote frequency in the obese group and the non obese is except for one study by **Yuan X et al.** almost identical.

Figure 2: Comparison of the distribution in % of the homozygote obese and controls



3. Discussion and Comparison

The aim of the current meta-analysis was to examine the possible association of the -759C/T polymorphism of the 5-HT2C receptor gene obesity.

But the assessed data didn`t associate the -759C/T polymorphism of the 5-HT2C receptor gene with obesity. This was verified in two ways: (a) the odds ratios of all the single studies and the pooled odds ratio with their confidence intervals were all except one single study not significant, and (b) the distribution of the T allele between obese and nonobese patients was observed and almost identical, also with just one runaway and this happened to be the same study again.

This very same study was conducted by **Yuan et al.** identifying the -759C/T polymorphism of the 5-HT2C receptor gene and showing an association of this polymorphism with obesity and T2DM [Yuan X et al., 2000]. The study, which included 123 obese patients, had some noticeable weaknesses, which could explain the disagreement to all other studies:

The first and most obvious is that the study population only contained only men, in the current meta-analyses I included men and women.

This disagreement could be also explained by the differences of the patients included in each study. In the meta-analyses, both men and women of Caucasian origin and Asian origen were included, with completely different ages. **Yuan et al**. studied the -759C/T polymorphism only in middle-aged men (mean age of 51 years), of ethnic Japanese origin.

In this case, differences in mean age of patients and in ethnicity might explain the disagreement in the findings between **Yuan et al**.`s study and the meta-analysis.

All studies show differences in the range body mass index for the obese and control groups:

In **Yuan et al**.`s study participants with an BMI ≥28 were matched to the obese group all below to the controll group. In the study by **Iordanidou M et al.** the obese group started with a BMI >30. **Kring SII et al.** defined obesity as 35% overweight relative to a local standard and this corresponds to a BMI ≥31.0 kg/m^2, which proved to be above the 99th percentile**. Bah J.et al.** on the other hand used a different definition of obesity, here the overweight group started with an BMI of > 25. Though all these BMI ranges are different, they can not explain the difference between **Yuan et al.**`s results and those of the other studies which are almost identical in their outcome, even if the BMI ranges are varying.

For a comparison I picked another association study by **Vimaleswaren KS et al**. from 2010 and to round it all up another meta-analysis by **De Luca et al.** form 2007.
The article “Association between serotonin 5-HT-2C receptor gene (HTR2C) polymorphisms and obesity-and mental health related phenotypes in a large population-based cohort” by **Vimaleswaren KS et al.** examined six HTR2C SNPs in 4978 men and women from the European Prospective Intervention into Cancer (EPIC)-Norfolk Study. To confirm borderline significant associations, the remaining 16003 individuals from the EPIC-Study were genotyped. Of the six SNPs, only the T allele of the -759 C/T SNP showed borderline significant association with a higher body mass index (BMI) (0,23 kg/m^2 , 95% confidence interval (CI): 0,01-0,44, p=0,051). As this association only achieved borderline significance the findings were validated in the remaining cohort. Here the association with the BMI remained borderline significant (0,20 kg/m^2, 95% CI: 0,04-0,44, p= 0,09).
A very similar field of research including the role of -759C/T polymorphism in body weight regulation is studied in schizophrenic patients receiving atypical antipsychotic agents, which induced weight gain [Miller D et al., 2005; Ellingrod VL et al., 2005; Reynolds CP et al., 2002]. Schizophrenic patients carrying the C allele were at higher risk to weight gain than those carrying a T allele and this finding suggested the protec-

tive role of the T allele for the development of weight gain during treatment with atypical antipsychotics.

It has been reported that the protective role of the -759T allele may result from decreased neuronal expression of the receptor, although it is possible that subsequent compensatory changes in other systems that control feeding mediate the resistance to antipsychotics- induced weight gain [Hill MJ et al., 2007]. On the other hand, there are several studies that have not replicated the role of the -759C/T SNP in the antipsychotics-induced weight gain [Tsai SJ et al., 2002; Basile VS et al., 2002; Theisen FM et al., 2004] and this SNP was not associated with the presence of the metabolic syndrome in schizophrenic patients [Mulder H et al., 2007].

These two opposing views are investigated in a meta-analysis by **De Luca et al.**:

In the Meta-Analysis "Association of the HTR2C gene and antipsychotic induced weight gain: a meta-analysis" **De Luca et al.** investigated the association of the HTR2C gene and antipsychotic induced weight gain combining all published data while restricting the analysis to studies investigating the 759C/T. Also ancestry (Caucasian vs. Asian) and clinical factors moderated any association were investigated.

Characteristics of included sample:

Study	n	Ancestry
Reynolds et al. (2002)	123	Asian
Tsai et al. (2002)	80	Asian
Basile et al. (2002a)	73	Caucasian, African-American
Müller et al. (2003)	59	Caucasian, African-American, Hispanic
Theisen et al. (2004)	97	Caucasian
Templeman et al. (2005)	73	Caucasian
Miller et al. (2005)	41	Caucasian, African-American, Hispanic
Ellingrod et al. (2005)	42	Caucasian

With these 8 studies they also found evidence for a slight association of -759C/T with weight gain and significance between studies for heterogeneity. This meta-analysis provides support for the association of HTR2C in weight gain but indicates that firmly establishing the role of pharmacogenetics in clinical psychiatry requires much larger sample sizes that have been hitherto reported.

After careful consideration of the assessed results from the conducted meta-analysis and the comparison other study in a large cohort and the second meta-analyses I suggest that the common HTR2C gene variants are unlikely to play a prominent role in obesity in the general population.

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Summary of epigenetic and genetic markers at the serotonin receptors in relation to personal traits and environmental triggers

1.Introduction:

Appetite could be in the simplest sense explained as a feedback system, but it is controlled by a complex combination of responses in the brain.
Hypothalamic centres has been extensively studied and been noticed to integrate information about long-term energy stores and other physiological and environmental factors to demand appropriate amounts of nutrition.
The brainstem mediates reflex satiety responses involving the sensing of short-term alterations in nutritional state causing the initiation of appropriate gastrointestinal and motor responses.
Serotonin is not only in the ideal position to coordinate or influence the responses of the neuraxis but it is also closely intertwined with many other simultaneously running processes. A significant amount of research has been conducted to characterize the interactions between serotonin and this food intake neurocircuitry.
Another very interesting and maybe beholding a lot of elucidating information, is the genetic and especially the epigenetic research in regard to serotonin. In this paper, however, I will take a step back and draw a greater picture, integrating not only the environmental triggers, but also introducing the life-style-choises, which are linked to the individual character and behavior in regard to appetite.
Here I will peripherally scratch pathological eating behavior, like bulimia nervosa, binge eating disorder und anorexia nervosa, which was from my point of view worth mentioning, because these disorders are linked not only to behavior and environmental influences, but also to epigenetic and genetic markers.

2. Epigenetic markers

Epigenetic models may play a promising new role of genes in appetite and especially all kinds of eating disorders. Epigenetic effects include alterations to the ways in which DNA functions within the cell. Such alterations to gene processing can change the function of gene products, for instance in serotonin receptors, transcribed from the DNA

without polymorphisms being present in the original gene sequence. These kinds of alterations are likely to be a major source of individual variability in genetically regulated aspects of eating and its pathology.

Histone alterations are already set up priorities of epigenetic research and involved in the regulation of gene expression [Szyf M et al., 2007]. There is some evidence that epigenetic alterations change both memory formation and fear conditioning [Miller CA & Sweatt JD, 2007], which are implicated in the pathogenesis and maintenance of eating disorders [Lascelles KRR et al., 2003].

Short excurse: Definition of Body dissatisfaction and eating disorders:

Body dissatisfaction

Not only adolescent females are particularly likely to report body dissatisfaction, also adolescent males experience body image disturbance as well. In general the nature of dissatisfaction seems to differentiate by gender, with the majority of women hoping to be slimmer and men equally split between those, who want to gain weight and those, who want to lose weight [Furnham A et al., 2002].

Kostanski and Gullone [Kostanski M & Gullone E, 1998] examined a nonclinical sample of adolescents and noticed that over 80% of females and 40% of males were highly unhappy with their bodies. Most females considered themselves as being two or more sizes too big than their ideal figure, sometimes even regardless of their actual body mass; on the other hand, adolescent males were divided fairly evenly between a desire to be larger and a desire to lose weight. Males' dissatisfaction was tied to body mass distribution, such that underweight males wished to gain muscle mass and overweight males wanted to be smaller and lose fat mass [Kostanski M & Gullone E, 1998]. Appearance has become a central evaluative dimension in Western cultures and has important influence on self-concept and self esteem [Harter S, 2002; Stice E & Bearman SK, 2001].

Eating disorders

Today we know a variety of different forms of eating disorders.

Anorexia nervosa generally appears in adolescence and is characterized by a severe restriction of food intake that results in weight loss to the point that the person has at most 85% of a healthy body weight.

Bulimia nervosa may not result in weight alterations but is characterized by unusual patterns of food intake that vary from periods of restriction to bingeing. Bulimia typically displays in adolescence or early adulthood and often after a period of dieting, which may or may not have resulted in weight loss [Kaye WH et al., 2000].

Last but not leased binge eating disorder is marked by recurrent periods of overeating without compensatory measures, like vomiting, frequently leading to rapid changes in weight and sometimes also leading to obesity.

Even though all eating disorders are very different, they are all characterized by intense body dissatisfaction and certain behavioral similarities.

Recent data also suggests epigenetic alterations in the reducing mRNA transcription of the 5-HT transporter (5-HTTT; [Philibert RA et al., 2008]), which is widely implicated in psychiatric disorders, like bipolar- and schizophrenic disorders, and certain behavioral traits of eating disorder pathology.

Taking a closer look at bulemia nervosa there was some evidence for epigenetic effects found on genes important for regulating serotonergic and dopaminergic function [Frieling H et al., 2008; Frieling H et al., 2009], although the functional impact needs further investigation.

An additional exciting part of the epigenetic research is that it also provides space for environmental triggers over gene transcription and processing.

For example, one model assumes that maternal behavior changes gene function in the hippocampus [Weaver ICG et al, 2006] and ultimately the hypothalamic–pituitary adrenal (HPA) stress response of individuals into adulthood [Liu D et al., 1997].

That leads to the justified assumption that if epigenetic effects are responsible for altering some processes of gene function in the 5-HT system, then individuals exposed or sensitive to epigenetic influences may be further influenced for elevated feeding and weight.

3. 5-HT receptor

The genetic and pharmacologic dysregulation of the serotonergic system is perhaps one of the most studied areas of research. The effects of 5-HT manipulation on feeding behavior have been demonstrated in both animal and human subjects [e.g., Blundell JE, 1986; Mancilla-Diaz JM et al., 2002; Soulairac A, 1963].

3. 1. 5-HT2a receptor

The 5-HT2a receptor is a postsynaptic receptor that elevates neuronal activation and is located in a number of cortical and prefrontal areas and particularly in the caudate nucleus, hippocampus, and nucleus accumbens [Barnes NM & Sharp T, 1999; Pazos A et al., 1987]. In response to prolonged 5-HT exposure the 5-HT2a receptor is downregulated [Sanders-Bush E, 1990].
Genetic polymorphisms of the 5-HT2a receptor have been found to be positively linked with novelty seeking, assuming that lower processing 5-HT2a receptors yield a higher likelihood of an approach response in the context of environmental uncertainty [Heck A et al., 2009].
In addition, higher density of the 5-HT2a receptor in the medial prefrontal cortex (mPFC) correlates with the coupling of mPFC–amygdala activation, connecting 5-HT2a functionality with emotionally relevant stimuli through cortico-limbic circuits [Fisher PM et al., 2009]. So the decreased functionality or density of 5-HT2a is likely to lead to elevated impulsivity or difficulty regulating affect [Frokjaer VG et al., 2008; Moresco FM et al., 2002], for example through functional polymorphisms [Nomura M et al., 2006].

3. 2. 5-HT1a receptor

5-HT1a receptor is an inhibitory autoreceptor in most of the times found on both, the presynaptic and postsynaptic neuron in the hippocampus, lateral septum, cingulated and entorhinal cortex, amygdala and raphe nuclei [Barnes NM & Sharp T, 1999]. Electrophysiologic studies confirm this inhibitory effect of 5-HT1a activation decreases cell firing in hippocampus [Kasamo K et al., 2001], forebrain [Ashby CR Jr. et al., 1994], and raphe nuclei [Haddjeri N et al 2004] neurons.

The inhibitory role of 5-HT1a over serotonergic transmission involves also anxiety-related phenomena. For example, diminished 5-HT1a binding is recognized in most anxiety disorders [Akimova E, et al. 2009]. In that way, most models of 5-HT1a dysregulations suggest a higher harm avoidance [Hansenne M & Ansseau M, 1999], although anxiety behaviors can occur with both under or overexpression of the receptor [Overstreet DH et al., 2003].
The effects of 5-HT1a neurons are also observed in the consolidation and reestablishment of emotional memory which is likely to play a major role in anxiety and mood disturbances [Drevets WC et al., 2007; Ogren SO et al., 2008].

4. Lifestyle Choices and Environmental triggers

4. 1 Chemicals

In addition to genetic and epigenetic mechanisms, environmental exposure to certain chemicals may add to difficulty with weight control and traits like compulsivity and impulsivity.
Several chemicals that manipulate an individual's ability to regulate and metabolize lipids, promote adipogenesis and rate or volume of fat deposition have been identified [Grun F & Blumberg B, 2009a, b]. It is believed that these chemicals like, phytoestrogens, synthetic estrogens, and environmental estrogens, act in metabolic processes early in life time and change one's ability to naturally keep a normal body weight. For example, even a low dose of bisphenol A (BPA) exposure, a substance found in plastics, leads to reduced fetal baby weight and subsequent estrous cycle dysregulation in offspring of rats [Rubin BS et al, 2001]. Application of BPA also yields elevated novelty seeking and approach behavior in rats, via dysregulation of the estrogen system [Gioiosa L et al., 2007] assuming that early dysregulation of the estrogen system may lead to trait-based deficits in inhibition and also alterations to basic metabolic processes.
These chemical effects seem to be functioning through estrogenic influence over enzymes that regulate glycolysis [Kostanyan A & Nazaryan K, 1992], insulin sensitivity [Kumagai S et al., 1993], mitochondrial structure and function [Justo R et al., 2005] and the tricarboxylic acid cycle [Yan et al., 2004].
Summarized, the exposure to different exogenous estrogenic agents can have a noticeable impact on energy metabolism and homeostasis [Chen JQ et al., 2009] or impul-

sivity, perhaps even inducing susceptibility in individuals to weight changes and so promoting early attempts at dieting or also the tendency for binge eating.

4. 2. Stress

Stress influences eating behavior and weight in both human and animal models. Stress in animals showed an elevated food consumption, particularly when the stressor is related to defeat experiences [Teskey GC et al., 1984]. A Gallup poll observed that 64% of Americans admitted to eat to relieve tension and negative emotion [Gallup G & Castelli J, 1989]. Humans often show a poorer impulse control during periods of high stress, so that short-term affect regulation frequently takes over self-regulatory goals [Tice DM et al., 2001]. Indeed, research confirms that people are more likely to eat greater amounts of sweet, high-fat foods when stressed [Oliver G et al., 2000]. Especially during adolescence, when various social, educational, and physical transitions can be stressful [Arnett JJ, 1999], the impact of stress on food intake maybe more sever. Self-reported levels of social stress find their peak during adolescence and early adulthood [Turner RJ, et al. 1995].

4. 3. Exercise and energy expenditure

Exercise is an important control of body weight and composition that plays a major role in producing and maintaining weight loss as well as preventing weight gain [Goldberg JH & King AC, 2006]. Even with no caloric restriction at all, weight loss can be achieved by increased physical activity [Ross R et al., 2000].
Many cross-sectional studies display that regular exercise is associated with lower rates of depression, neuroticism, and anxiety at almost all ages, too [Moor MHM et al., 2006]. Some of these effects appear via mechanisms similar to those underlying antidepressants: through activation of monoaminergic transmission in different brain areas [Russo-Neustadt AA et al., 2004]. For instance, physical exercise elevates both synthesis and metabolism of serotonin in the hypothalamus and hippocampus [Dey S et al., 1992], which leads to a general well-beeing.

4. 4. Dieting

Although dieting is generally stimulated by a desire to lose weight, it is associated not only with weight loss but also with weight gain. Intense dieting and weight loss efforts have actually been shown to make especially adolescents more vulnerable for gaining weight, even after controlling for initial body mass [Field AE et al., 2003; Neumark-Sztainer D et al., 2006; Stice E et al., 1999].

There are at least two fundamental explanations existing for this relation:

First, dieting is considered to lead to greater metabolic efficiency, which means that the body requires fewer calories over time to maintain the same weight [Leibel RL et al., 1995; Wadden TA et al., 1990].

Second, in the early stages of the diet the resitance is too low to sustain over time, making overeating and weight gain increasingly likely [Polivy J & Herman CP, 2002].

Table 1: Effects of discussed factors

Developmental factor	Relation to appetite	Relation to weight
Exercise	+	−
Body dissatisfaction		+/−
Dieting	+/−	+/−
Eating disorders	+/−	+/−
Stress	+/−	+/−

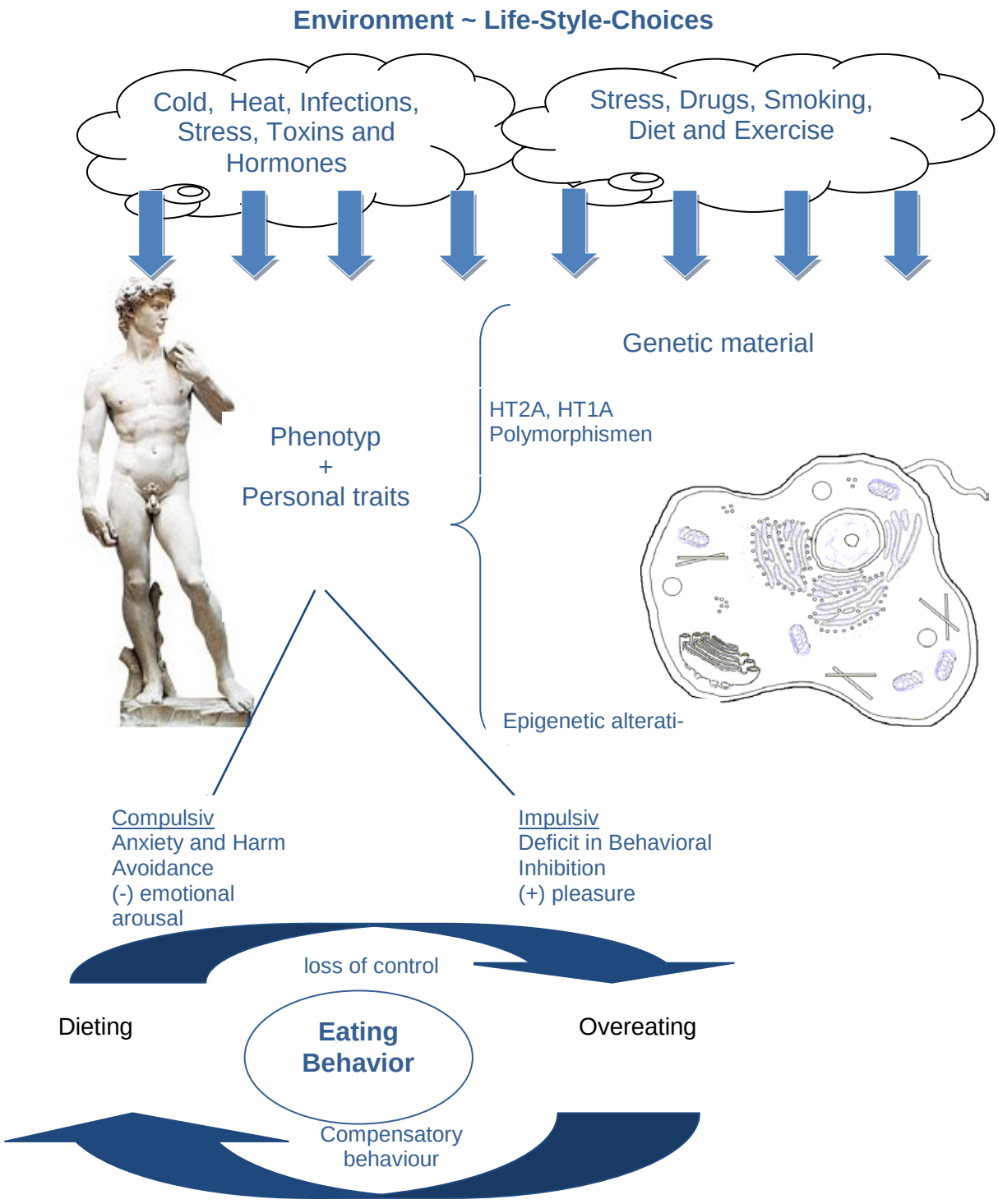
5. Concusion

On the basis of extensive genetic and pharmacological evidence, serotonin plays an important role in the control of food intake and, consequentially, body weight. The serotonin system is extremely complex in terms of anatomical projections, receptor subtypes, and its broad functional and influential roles. Nevertheless, ongoing research seems only to continues to scratch the surface of brain pathways underlying the regulation of food intake and body weight by brain serotonin. Much remains to be understood about the serotonic system and its influence in food intake control, the specific roles of each of the serotonin receptor subtypes involved, and the nuances of the effectors pathways.

The availability of genetic techniques and research permitting fine control of gene expression is likely to be of particular importance in the further understanding of the mechanism through which serotonin influences food intake. Greater knowledge about serotonergic mechanisms affecting food intake is likely to lead to more efficient serotonin-based pharmacotherapies to aid in appetite control in obese individuals.
Currently, there is only one serotonergic drug, the selective 5- HT2CR agonist lorcaserin, in late stage clinical development for the treatment of adiposity. However recent research assumes that a combination therapie, targeting multiple serotonergic receptors or other feeding-related pathways, may be more beneficial.

The closing illustration should ones again remind the reader that serotonin does not stand on its own, but is a “global-player” influencing and being influenced by many “markets”.

The figure below should highlights the series of factors in which the serotonergic systems an important factor in the normal and diseased development of eating behavior. Genetic polymorphisms from birth can reduce the efficacy of the serotonergic system. After birth, specific forms of stress brought about by parenting style may further compromise serotonergic genes. Simultaneously, the individual is exposed to environmental estrogens, which maybe makes the individual more vulnerable for weight gain and higher levels of subcutaneous fat deposition.
Puberty brings a rapid surge of hormones, like estrogens and testosterone. Social pressures to be thin or have perfectly defined body increase and the following consequence of these pressures may include increased attempts at dieting, but also more severe patterns can occur like bingeing, and purging. This pattern will persists until the individual is able to harmonize the pattern and find an own balance of eating and exercise.


Environment ~ Life-Style-Choices
Cold, Heat, Infections, Stress, Toxins and Hormones
Stress, Drugs, Smoking, Diet and Exercise
Genetic material
HT2A, HT1A Polymorphismen
Phenotyp + Personal traits
Epigenetic alterati-
Compulsiv
Anxiety and Harm Avoidance
(-) emotional arousal
Impulsiv
Deficit in Behavioral Inhibition
(+) pleasure
loss of control
Dieting
Eating Behavior
Overeating
Compensatory behaviour

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